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Visualization of Hydrogels with Variable-Pressure SEM

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Hydrogels are an important class of biomaterials with exceptional promise in biomedical applications [1,2]. Their use in reconstructive surgery, bioadhesives and as controlled drug release devices additionally do not incur the typical immunological reactions that occur with living organ transplants [3]. They are water-swollen, cross-linked polymeric structures containing either covalent bonds produced by the simple reaction of comonomers, physical cross-links from entanglements, association bonds or strong van der Waals interactions [4, 5]. The physical behavior of hydrogels is dependent on their equilibrium and dynamic swelling behavior in water. Knowledge of their swelling characteristics is of utmost importance in biomedical and pharmaceutical applications since the equilibrium degree of swelling influences not only the solute diffusion coefficient, but also surface and optical properties, and surface mobility. Determining hydrogel characteristics with visualization techniques under hydrated conditions is therefore imperative for biomedical application.

Various imaging techniques have been used to determine hydrogel surface structure, with reports on SEM indicating severe surface aberrations caused by either the dehydrating techniques required by high-vacuum SEM, or damage from cryo-SEM [6]. The need for dehydration and critical point drying, or freeze-drying of hydrated samples in SEM imaging, generally limited the application of SEM for hydrogel imaging, with AFM often the preferred technique. However, the development of Variable-Pressure SEM has enabled visualization of hydrated samples, albeit for limited periods of time due to the gradual loss of water under low pressure. Maintaining a higher chamber pressure (250Pa) to prevent moisture loss limits resolution, while, on the other hand, the decrease of chamber pressure to improve resolution limits imaging time by increasing moisture loss.

I here describe the development and application of a technique for high-resolution imaging of hydrogels under lower pressure conditions (50-60Pa) by limiting moisture loss through control of stage temperature. Scanning electron microscopy was carried out with a Hitachi S-3400N VP-SEM and temperature controlled with a Deben Peltier coolstage. Samples are mounted in a thin film of water on a 10mm stub fitting the coolstage. Conditions are controlled to limit water-loss while optimizing resolution. Initial stage temperature is set at 4°C, and decreased to -10°C until a controllable pressure (270Pa) is reached. Pressure and temperature are then correlatively decreased (Fig.1), until a chamber pressure of 60Pa, and correlated stage temperature of -25°C is reached. High-energy backscattered electrons at 15kV provide the required signal for high-resolution imaging. Decreasing temperature too rapidly may result in the formation of obstructive ice crystals. Salts from retained buffer may have a similar effect when dehydration occurs if pressure is decreased too rapidly

The distribution of embedded nano-particles (Fig.2), consistency of hydrogel pore size (Fig.3), and hydrogel structure comparing various drying and freezing methods, was carried out repeatedly, without application of any chemical or physical procedures that may interfere with the intrinsic hydrogel structure. To visualize cell growth on hydrated biomaterial (Fig.4), a heavy-metal biological stain (OsO_4), providing an increased number of backscattered electrons

due to its high atomic number, was applied after primary fixation with 2% Glutaraldehyde and 4% Paraformaldehyde. Post-fixation with OsO_4 not only stabilized structure, but also enhanced contrast, and thus cell detection, under hydrated conditions.

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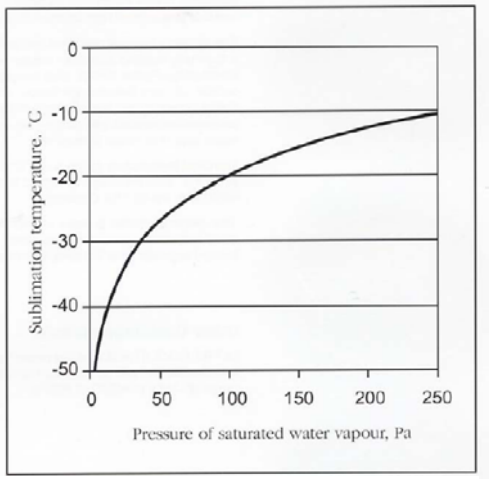

Fig.1 Correlation of sublimation temperature and pressure of saturated water vapor

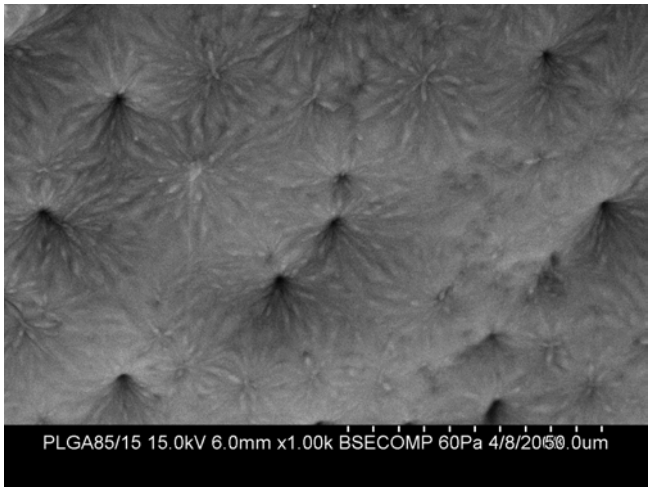

Fig. 2 Distribution of embedded nanoparticles in hydrogel

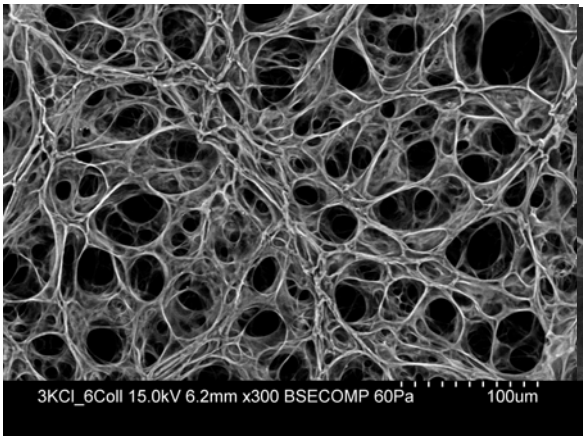

Fig.3 Investigating parameters influencing pore size of hydrogels.

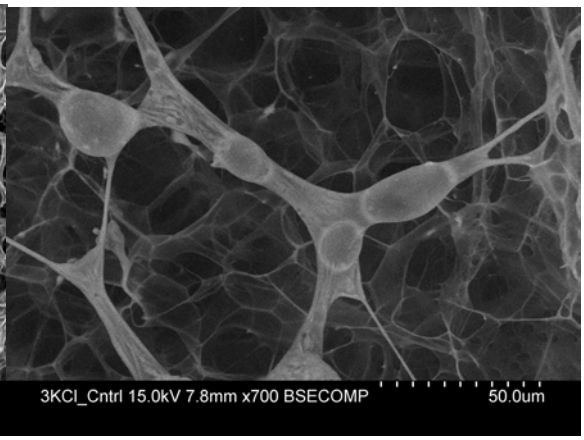

Fig.4 Cell growth on hydrogel fixed with Osmiumtetroxide

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